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Different tolerances to chemical contaminants between unicellular and colonial morph of *Microcystis aeruginosa*: Excluding the differences among different strains

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HIGHLIGHTS

- Different tolerances to contaminants between unicellular and colonial *Microcystis* were analyzed.
- The differences in the physiology among different strains were excluded.
- The cell density, cell viability, superoxide dismutase, and malonaldehyde were analyzed.
- Colony formation of *Microcystis* could be considered as a strategy in response to chemical stress.

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ABSTRACT

In order to ascertain the different tolerances to chemical contaminants in one strain of *Microcystis* with different morphology, unicellular and colonial *Microcystis* in one strain was obtained from different conditions of light intensity and temperature. The samples were divided into 8 groups including control (no chemical addition), $CuSO_4$, chloromycetin, and linear alkylbenzene sulfonatelas (LAS) treatments. The cell density, cell viability, superoxide dismutase (SOD), and malonaldehyde of *Microcystis* were analyzed. It was observed that cell density of both unicellular and colonial *Microcystis* increased from the beginning to day-5 in the control and the $CuSO_4$ treatments. However, the growth of *Microcystis* was significantly inhibited in the culture with chloromycetin and LAS treatments. Notably, the inhibition rate was significantly high in unicellular *Microcystis* relative to the colonial *Microcystis*. The esterase activity in all the treatments decreased dramatically relating to the control. In addition, the esterase activity in colonial *Microcystis* was significantly higher than that of the unicellular *Microcystis* in all the treatments. Although there were no significant differences in activities of SOD between the two morphologies in the control treatments, in all the other treatments, significant differences were observed. The results proved that colony formation of *Microcystis* could be considered as a strategy in response to chemical stress.

1. Introduction

Microcystis is a common harmful bloom-forming algae in freshwater eco-systems worldwide [1]. The microcystins (MCs) produced by *Microcystis* seriously threatens the regional drinking water safety around the world [1,2]. Moreover, *Microcystis* blooms can also cause unpleasant odor [3], depletion of oxygen resulting in death of fishes [4], and some other ecological problems [5].

Microcystis normally occurs as single cells in axenic cultures in the laboratory as a result of a long-term cultivation [6,7], but forms colonies under natural conditions [8,9]. Colonial morph was reported to be important in blooms formation of *Microcystis* [10] because it can affect floating velocity [11,12] and anti-predator defence [13,14] of *Microcystis*.

Moreover, colony formation was reported as a good inherent strategy to reduce the damage from adverse factors [15]. It was revealed that colonial morph has the potential to resist the damage caused by *Microcystis* inhibiting substances [16,17]. Colony

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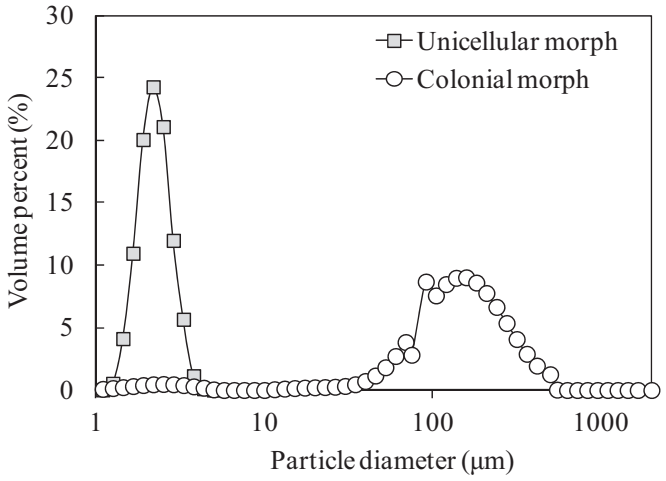

Fig. 1. Size distribution of unicellular and colonial *Microcystis* obtained from different culture conditions.

formation of *Microcysits* also provides an efficient strategy for protection against virus-induced mortality [18]. According to Wang et al. [19], the algicidal bacterium *Pseudomonas aeruginosa* ACB3 could inhibite the growth of unicellular *Microcystis* strains effectively but hardly inhibit the growth of colonial strains. Batch culture experiments also demonstrated that colonial *Microcystis* grew better under nutrient and iron limited conditions than unicellular *Microcystis*. Besides, colonial morph of *Microcystis* can also reduce the occurrence of photoinhibition under high light intensities [20,21].

However, most of these results were speculated from culture experiments with different *Microcystis* strains. Thus, the differences in physiological property among different strains (unicellular or colonial morph) cannot be excluded. Our previous study [22] demonstrated that unicellular *Microcystis* would form large colony at low specific growth rate induced by low light intensity and temperature. Therefore, it provided the possibility to exclude the differences among different strains by culturing unicellular *Microcystis* under conditions with low light intensity and temperature.

The aims of this study are (i) to reconsider the different tolerances to chemical contaminants between unicellular and colonial morph of *Microcystis* excluding the differences among different strains (ii) to prove that colony formation of *Microcystis* could be considered as a strategy in response to chemical stress. To exclude the differences among different strains, a unicellular *Microcystis* strain was cultured under conditions with different light intensity and temperature. The size distribution of *Microcystis* cultured under different conditions showed that both unicellular and colonial morphological *Microcystis* were obtained from the same strain. Thus, the toxicological tests carried out in the current study can evaluate the tolerances to chemical contaminants of unicellular and colonial morph of *Microcystis* excluding the differences among different strains.

2. Materials and methods

Unicellular *M. aeruginosa* (Kützing) Kützing strain FACHB 469 was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. The algae were batch-cultured axenically in 150 mL of sterilized liquid BG-11 medium in a 250 mL conical flask under a 12:12 h light–dark cycle. The initial cell density of *M. aeruginosa* was 5×10^4 cells mL^{-1}. To obtain different morphological *Microcystis*, the algae were batch-cultured under two different condition with different light intensity and temperature: (i) 50 μmol photons $m^{-2}\,s^{-1}$ at 25 °C for 9 days (sample A – unicellular morph); (ii) 15 μmol photons $m^{-2}\,s^{-1}$ at 20 °C for 15 days (sample B – colonial morph). Three parallel samples were considered for each condition.

After the cultivation, a bottle of samples A and B was used to analyze colony size and cell density. Colony size was analyzed by a laser particle analyzer, and cell density was analyzed by cell counting method [22].

The rest of the samples were then transfered under the condition (i). The samples were divided into eight groups (A1–A4, B1–B4). Each group had three parallel samples. Groups A2 and B2 were enriched with $CuSO_4$ to yield total copper concentration of 0.25 mg L^{-1}. This concentration was always used to control the nuisance algae blooms [16]. Groups A3 and B3 were treated with chloromycetin, and its concentration was adjusted as 10 mg L^{-1} which induced a 90% inhibition rate [23]. Groups A4 and B4 were treated with linear alkylbenzene sulfonatelas (LAS, obtained from Sigma Chemical Company (USA) with a chemical formula of $C_{18}H_{29}NaO_3S$), and its concentration was adjusted to 100 mg L^{-1} which also induced a 90% inhibition rate [15]. Groups A1 and B1 were set as control group without any treatment.

The cell density of each sample was analyzed everyday. At day 5, cell viability (analysed by 2,3,5-triphenyltetrazolium chloride (TTC) reduction), superoxide dismutase (SOD), and malonaldehyde (MDA) were analyzed according to the methods described by Hong et al. [24], Choo et al. [25], and Kong et al. [26].

The growth curve was presented as mean, whereas the maximum and minimum were presented as error bars. The other data were presented as mean ± SD. Significant differences were determined by analysis of variance (ANOVA) using tukey post hoc test via SPSS 10.0.

3. Results and discussion

Fig. 1 shows the size distribution of unicellular and colonial *Microcystis* obtained from different culture conditions. The particle size of unicellular *Microcystis* was from 1.5 to 3.8 μm. The size of colonial *Microcystis* ranged from 50 to 500 μm. The mean particle size of unicellular and colonial *Microcystis* was 2.7 and 133 μm, respectively.

The growth curves of unicellular and colonial *Microcystis* with different treatments are depicted in Fig. 2. The initial cell density of unicellular and colonial *Microcystis* was 1693×10^4 and 762×10^4 cells mL^{-1}, repectively. In the control culture, cell density of both unicellular and colonial *Microcystis* increased from the beginning to day-5. The maximum value of the cell density of unicellular and colonial *Microcystis* reached 3401×10^4 and 2895×10^4 cells mL^{-1}, respectively. The growth curves in the culture treated with 0.25 mg L^{-1} Cu^{2+} were similar to those in the control, but the maximum cell density of unicellular and colonial *Microcystis* was just 2472×10^4 and 2313×10^4 cells mL^{-1}, respectively. The growth of *Microcystis* was significantly inhibited in the culture treated with 10 mg L^{-1} chloromycetin and 100 mg L^{-1} LAS. The cell density increased for 4 and 3 days, respectively, and then decreased subsequently.

Table 1
The inhibition rate to both unicellular and colonial morphological *Microcystis* with different treatments.

Treatment	Cu^{2+}	Chloromycetin	LAS
Unicellular morph	28.1	63.3	74.0
Colonial morph	20.1*	50.4**	64.3**

* Indicates that differences between unicellular and colonial morph are significant at $P < 0.05$ level.
** Indicates that differences are significant at $P < 0.01$ level.